THE TOTAL PCB-TASK: A COMPREHENSIVE HRGC-HRMS METHOD FOR ANALYSIS OF ALL 209 PCB CONGENERS IN REFINED FISH OILS

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Introduction

Polychlorinated Biphenyls (PCB) have been known as being toxicologically relevant for a long period of time. There are different toxicological action modes of PCB, comprising dioxin-like (dl) and non-dioxin-like (ndl) activities, depending on specific chlorination patterns of the respective congeners. Derived from these facts, there have been ongoing discussions and activities in order to minimize human PCB uptake as far as possible and to regulate maximal allowed levels and intakes. One of these activities resulted in the "safe harbour levels" for PCB, issued by the Californian government in legislative Proposition 65[1]. This issued level describes a maximum daily intake considered as being safe for human health, expressed as "total PCB". This legislative demand resulted especially for food additives as e.g. otherwise healthy fish oil products in a question about their PCB content. It therefore implies the ability of precisely analysing the "total PCB"-Content in terms of determining all 209 PCB congeners. There have been several different approaches for respective methods and calculation modes, reaching from quantification against technical PCB mixtures over fractionation of PCBs to complete generic templates as e.g. US-EPA method 1668[2]. All these approaches led to incomplete or economically problematic analytical methods. We present a completed approach using a specialised multistep-cleanup together with a modern HRGC column which enables a comprehensive analysis of all PCB with 175 peak separations. The method has been applied on refined or semi-refined fish oils of south American origin from different fish species (anchovy, tuna) which are intended for dietary supplement use.

Materials and methods

The analysed samples were taken from the routine input of refined fish oils and fish oil derivatives over a period of 3 months (Feb. to April 2012). The samples have been analysed for total PCB and all PCB homologue groups by analysis of all 209 PCB congeners from Mono- to Decachlorobiphenyl(s) with determination of single compounds, including the 12 dioxin-like PCB (dl-PCB, "WHO-PCB"; IUPAC# 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189). Additionally, calculations of TEQ-values and congener group totals have been performed.

All analytical data are reported in pg/g product, all TEQ values are given as upperbound TEQ using WHO-TEF(1998). The analyses have been performed at the Eurofins GfA GmbH Dioxin/POPs competence centre, Hamburg. For sample preparation, 3 g sample material has been dissolved in n-hexane prior to further processing. The applied clean-up consisted of multiple column chromatography with acidic silica and basic aluminium oxide columns and an adapted elution in order to collect all PCB congeners completely within one fraction without losses violating the set quality criterion (see below).

GC separation was achieved on a 60m *0.25 mm i.d. * 0.25 µm d_f SGE HT8PCB column with unique properties. The SGE HT8PCB column has a high peak separation capacity for PCB but is reported to be temperature sensitive e.g. on transport or storage, irreversibly changing its properties[3]. Therefore, a specific *modus operandi* had to be established, since elution profiles for the 209 PCB deviated from the specified[4] elution order. The used GC column was comprehensively examinedfor the actual PCB congener elution order by application of standard solutions for all 209 PCB congeners. Under the given condition, a total of 175 separations were established as being suitable for project requirements. As an example, the separation for the main Hexachlorobiphenyls (HexaCB) is shown (fig.1). We defined 50% valley as a peak separation criterion on a mid-point calibration standard solution in combination with a review of typical PCB occurrences in biological (viz. fish) matrices. This has been done in order to avoid reporting of closely eluting congeners which are – due to major concentration differences – not evaluable under practical conditions (e.g. PCB 102 would practically be

not evaluable besides 93/95/98). By accepting less severe quality criteria and higher effort on evaluation of real sample patterns, the presented method allows for up to 187 peak separations.
Detection was performed by a state-of-the-art HRMS method on a Thermo DFS mass spectrometer at mass resolution $r \geq 10.000$.

Calibration was established with an initial multi-level curve as reference and employing daily single-point calibrations, counter-checked against the multi-level calibration. This has been performed individually for all reported congeners/congener groups. Quantification was performed by an isotope dilution method using a set of 35 ^{13}C-labelled quantification standards added before extraction (including first/last-eluters, 12 dl-PCB, 6 marker-PCB). Care has been taken to quantify all analytes by quantification standards from their own chlorination degree as far as technically possible with HRMS registrations of not more than 4 PCB chlorination degrees at the same time. For QA/QC, recovery rates have been monitored with 7 ^{13}C-labelled standards added prior to GC/HRMS injection. Recovery rates were accepted between 40 and 130% for all, or 50-120% for 90% of the isotope labelled standards respectively. Average recovery rates for the quantification standards were stable at (69±12)%, values below 40% occurring at less than 1%.
Further QA/QC measures consisted of having batch blanks prepared over the whole procedure as well as reference samples. The limit of quantification was established based on averaged blank values.

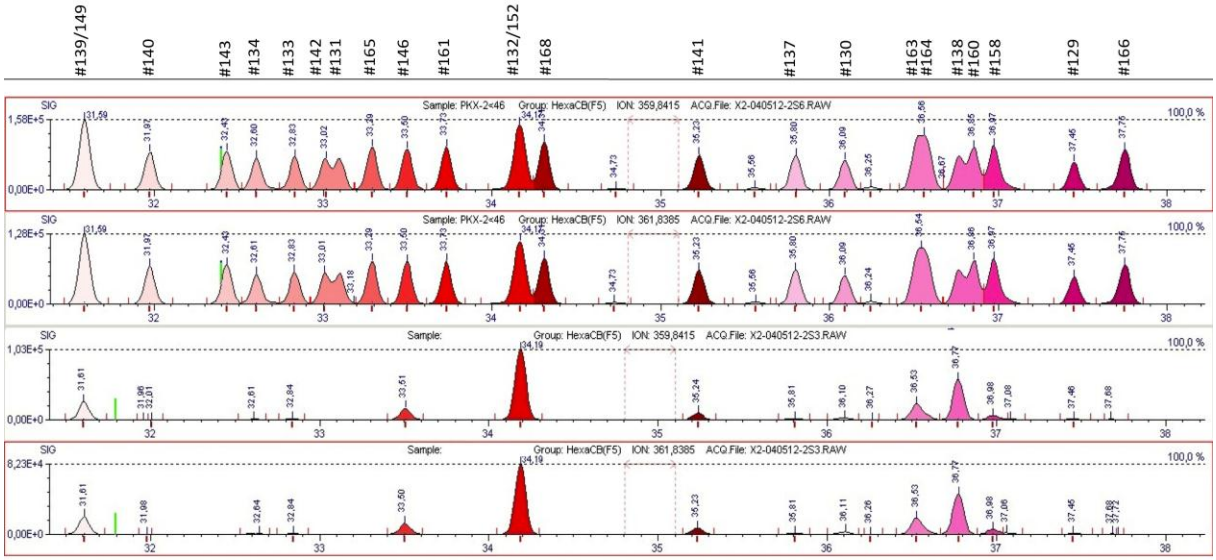

Figure 1: Separation for the main Hexachlorobiphenyls (HexaCB) congeners on a modified HT8PCB-column. Upper two traces: calibration standard, lower two traces: fish oil sample

Results and discussion:
A total of 25 fish oil samples have been analysed for PCB in the course of this developmental work between February and April 2012. They comprise two groups of refined or partially refined fish oils, anchovy oils (Engraulidae *spec.*, n=19) and tuna oils (Scombridae *spec.*, n=6). The results for the main PCB congeners and congener groups are shown in table 1. The found PCB concentrations are generally lower than general levels found for fish oil[5], regarding TEQ values. The total PCB concentration is at an average of 885 pg g^{-1} for anchovy oils and 3348 pg g^{-1} for tuna oils, respectively with maximum values of 7809 pg g^{-1} (anchovy oil) and 16275 pg g^{-1} (tuna oil). The maxima of the congener group distribution are at the Hexachlorobiphenyls (HexaCB) for anchovy oil and Heptachlorobiphenyls (HeptaCB) for tuna oil.

	anchovy oil, refined							tuna oil, refined						
	average	median	min	max	SD	rel. SD	n	average	median	min	max	SD	rel. SD	n
			pg/g fw			%				pg/g fw			%	
totals MonoCB	-	-	-	-	-	-	0	17,9	17,9	4,2	31,7	19,5	109%	2
totals DiCB	17,6	20,0	4,7	25,0	7,1	40%	7	60,0	60,0	18,2	101,8	59,1	99%	2
totals TriCB	11,7	12,7	2,5	26,2	9,7	83%	5	435,0	435,0	435,0	435,0	-	-	1
totals TetraCB	7,4	2,7	1,0	40,9	12,7	172%	9	1696,2	1696,2	1696,2	1696,2	-	-	1
totals PentaCB	110,5	37,0	16,2	875,4	223,2	202%	14	716,6	24,8	2,3	4194,7	1704,0	238%	6
totals HexaCB	418,7	128,3	6,5	4150,2	934,3	223%	19	1213,8	328,0	35,7	6166,8	2431,2	200%	6
totals HeptaCB	279,2	109,0	21,5	2355,0	532,0	191%	19	792,6	475,3	46,8	3080,9	1146,0	145%	6
totals OctaCB	49,2	23,0	2,3	340,5	78,6	160%	19	151,0	123,7	24,3	437,9	151,5	100%	6
totals NonaCB	6,0	4,2	1,5	18,7	4,6	77%	16	28,4	26,4	4,0	68,6	23,5	83%	6
DecaCB	3,0	2,6	1,9	7,0	1,3	43%	17	19,4	20,2	6,6	33,5	9,4	49%	6
total PCB (Mono-Deca)	849,2	269,2	46,9	7785,3	1745,5	206%	19	3303,1	1009,2	128,1	16247,1	6359,4	193%	6
WHOTEQ98 upperbound	0,18	0,09	0,05	1,28	0,28	155%	19	0,85	0,36	0,10	3,31	1,23	145%	6

Table 1: PCB homologue groups and totals in fish oil samples of the South Atlantic

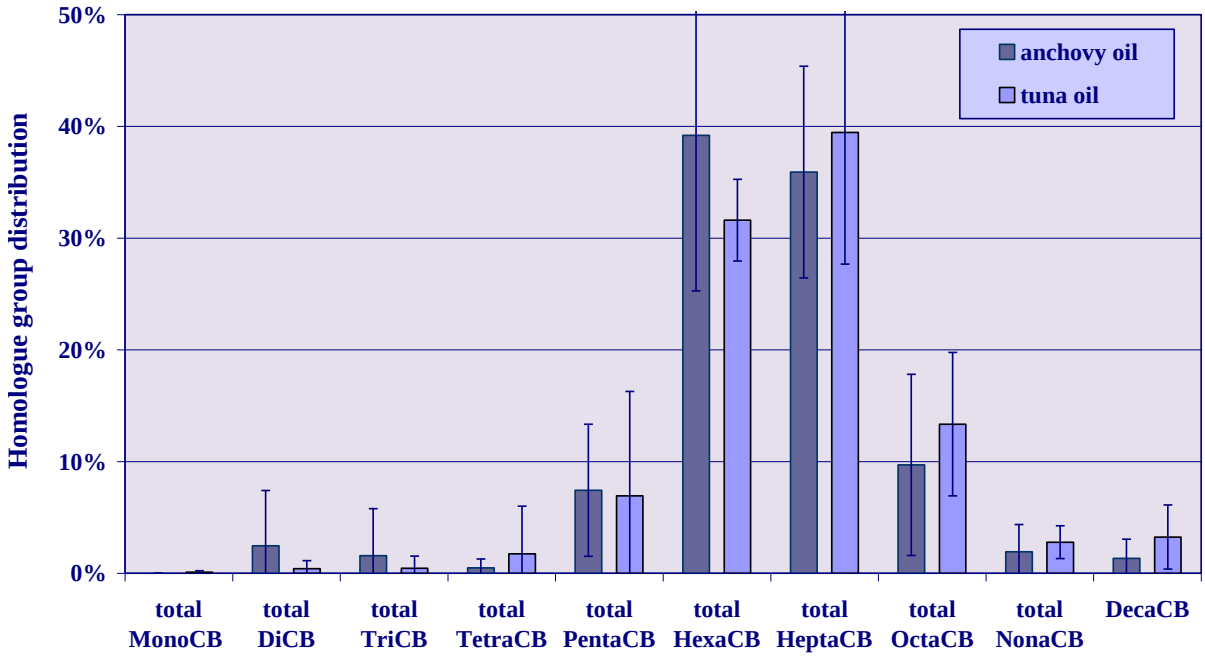

Figure 2: PCB homologues distribution pattern for refined anchovy oil (n=19) and tuna oil (n=5) samples

The fish oil samples analysed for this study show a relatively uniform distribution of PCB homologue profile with a maximum at the Hexa- to HeptaCB. This reflects the contribution of higher chlorinated technical PCB mixtures as e.g. Clophen A60 or Arochlor 1260[5] to global PCB pollution as well as metabolic pathways and loss of the more volatile PCB congeners.

Further discussions of homologue distributions as well as profiles of individual congeners are needed since they can be affected by different factors as e.g. different refining steps and refining grades of fish oil production or different origin of individual samples and sample groups. Also, the two fish families are at different trophic levels (anchovy at 3, tuna at 4) and may show different intake and metabolism of PCBs. Seeing this, an exact characterisation of the samples is needed for future work,

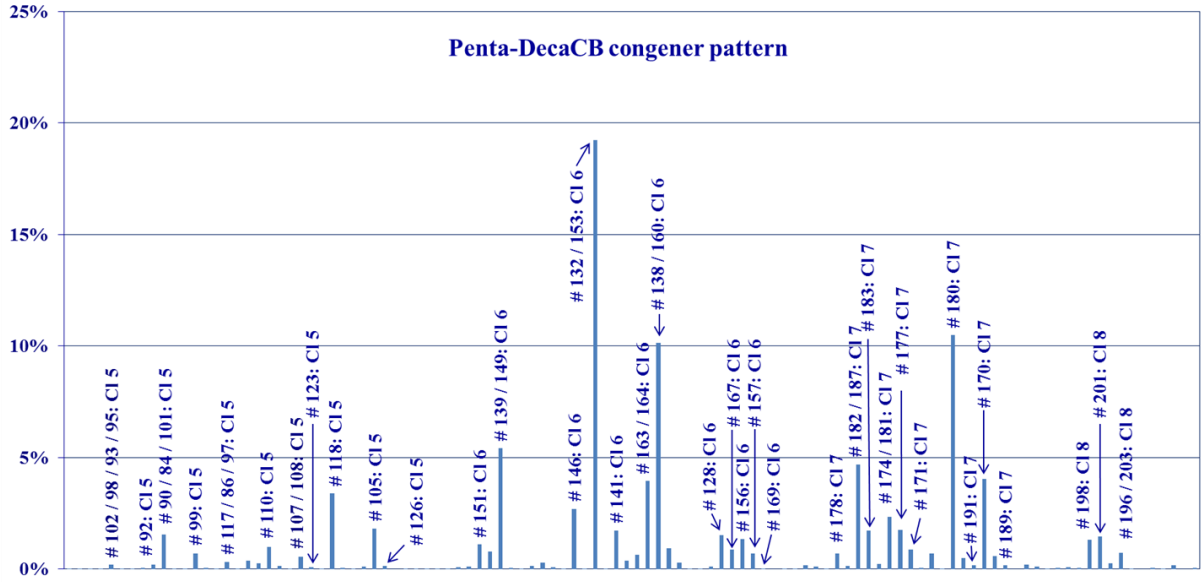

Figure 3: Example of the single PCB congener distribution (Penta- to DecaCB) of fish oil
Annotation: Congener:Chlorination degree

The development of an analytical method for PCB besides of "only" separating 18 main compounds, viz. dl-PCB and marker-PCB, will in every case remain a formidable task where compromising is necessary due to co-elution of peaks and overlap of homologue groups. An analysis of PCBs under the given conditions remain to have issues of minor – or at worst: moderate – importance. Care should be taken that results are not excessively biased by fragment signals from co-eluting PCBs of other chlorination degrees; also the secondary Dichlorobiphenyl mass is compromised at lower concentrations by the background of FC5311 used as mass reference compound ("lock mass"). These disadvantages can be dealt with by establishment of adapted quality criteria or on a project-by-project base and only exceptionally affect results in a significant way. The obtained information from analysis of altogether 25 fish oil samples gives further possibilities for studying patterns and single congener distributions as well as it poses analytical questions, e.g. regarding evaluation and judgement of incomplete or biased homologue patterns.

References:

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5. *Results of the monitoring of dioxin levels in food and feed, EFSA Journal 2010; 8(3):1385*
6. *Ishikawa Y., Noma Y., Mori Y, SakaiS: Congener Profiles of PCBs and New Proposal of Indicator Congeners, Organohalogen Compd. 2004 (66)*